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(54) Title: DERIVATIVES OF CYCLODEXTRINS EXHIBITING ENHANCED AQUEOUS SOLUBILITY AND THE USE THEREOF (57) Abstract Sulfoalkyl ether cyclodextrin derivatives and their use as solubilizing agents for water insoluble drugs for oral, intranasal, or parenteral administration are disclosed.		

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Description

Derivatives of Cyclodextrins Exhibiting Enhanced Aqueous Solubility and the Use Thereof

Technical Field

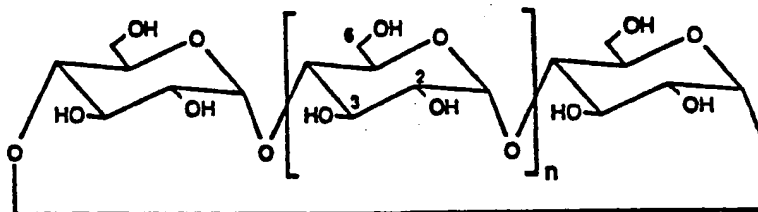
5 The present invention relates to cyclodextrin derivatives and to their pharmaceutical application as clathrating agents.

Background Art

Cyclodextrins (CD) are a group of cyclic homologous
10 oligosaccharides that are obtained from the degradation of starch by the action of the enzyme cyclodextrin transglycosylase elaborated by the bacterium Bacillus macerans. Published methods exist for the production of cyclodextrin transglycosylase as well as making and
15 isolating the cyclodextrins.

Cyclodextrins are cyclic molecules containing six or more α -D-glucopyranose units linked at the 1,4 positions by a linkages as in amylose. As a consequence of this cyclic arrangement, the molecule is characterized as having
20 neither a reducing end group nor a non-reducing end group.

The molecule is represented below by schematic formula (1) where the hydroxyl groups are shown in the 2, 3, and 6-positions of the glucopyranose units.



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Variable n may be a number from 4 to 6, or higher.

When $n=4$ the molecule is commonly known as α -cyclodextrin or cyclohexaamylose, when $n=5$ the molecule is commonly known as β -cyclodextrin or cycloheptaamylose and when $n=6$ 5 the molecule is commonly known as γ -cyclodextrin or cyclooctaamylose. When reference is made here to "cyclodextrin", it is intended to include the foregoing forms of cyclodextrin as well as molecules where $n>6$.

It is believed that as a consequence of the cyclic 10 arrangement and the conformation of the α -D-glucopyranose units, there is limited free rotation about the glycosidic bonds, and the cyclodextrins exist as conical shaped molecules with the primary hydroxyls situated at the small end of the cone and the secondary hydroxyls situated at the 15 large opening to the cone. The cavity is lined by hydrogen atoms from C_3 and C_5 along with the glucosidic oxygen atoms resulting in a relatively lipophilic cavity but hydrophilic outer surface.

As a result of the two separate polar regions and the 20 changes in solvent structure that occur upon complexation, cyclodextrins have the ability to form complexes with a variety of organic and inorganic molecules. The formation of cyclodextrin inclusion complexes with molecules is referred to as the "host-guest" phenomenon.

25 These unique properties of cyclodextrins have resulted in their commercial application in agriculture, water treatment, as surfactants and in drug delivery systems. The application of cyclodextrins in the pharmaceutical field has resulted in time release micro encapsulation,

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improved stability, and increased aqueous solubility of various drugs.

Cyclodextrins are known generally to improve the dissolution rate of drugs. The complexes formed are, however, also stable in aqueous solution, so that the improvement in dissolution is accompanied by an increase in the saturation solubility of the drug. Unfortunately the very β -cyclodextrin that forms the most stable complexes with most drugs has the lowest water solubility, so that drugs that are complexed with it cannot be brought into solution at therapeutic concentrations. The reason for this appears to be due to the crystalline structure of β -cyclodextrin itself.

Chemical modification of cyclodextrins is known to modulate their properties. Electroneutral cyclodextrins have been described by Parmerter et al (U.S. Patent No. 3,453,259), and Gramera et al (U.S. Patent No. 3,459,731). These are obtained by the condensation reaction of cyclodextrins with various epoxides or organic halides.

Other derivatives include cyclodextrins with cationic properties (Parmerter (I); U.S. Patent No. 3,453,257), insoluble crosslinked cyclodextrins (Solms; U.S. Patent No. 3,420,788), and cyclodextrins with anionic properties (Parmerter (II); U.S. Patent No. 3,426,011). Among the cyclodextrin derivatives with anionic properties, carboxylic acids, phosphorus acids, phosphinous acids, phosphonic acids, phosphoric acids, thiophosphonic acids, thiophosphinic acids, and sulfonic acids (see Parmerter (II), supra), have been appended to the parent cyclodextrin.

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Cyclodextrins have found applications in pharmaceutical delivery systems. As a "host" for "guest" drug molecules, these inclusion (clathrate) complexes have shown increased aqueous solubility for pharmaceuticals with intrinsically low aqueous solubility (Jones; U.S. Patent 4,555,504).

This solubilization results in the improved bioavailability for some drugs. As a clathrate complex some drugs have shown improved chemical stability in aqueous solution (Harada et al; U.S. Patent No. 4,497,803 and Hayashi et al; U.S. Patent No. 3,816,394). In addition, cyclodextrins have proved effective in controlling the release of highly water soluble pharmaceuticals (Friedman; U.S. Patent 4,774,329).

Despite this pharmaceutical utility, cyclodextrins are not without their limitations. The use of cyclodextrins in the clinical setting is limited to oral and topical dosage forms as the cyclodextrins exhibit nephrotoxicity upon entering the body unmetabolized. Since mammalian enzymes are specific for the degradation of linear starch molecules, the cyclodextrins remain largely unmetabolized and accumulate, due to their recirculation and readsorption, in the proximal tubule cells.

Cyclodextrins and their derivatives are mostly crystalline solids and concentration in the renal tissue is followed by crystal formation causing necrotic damage to the cells. Despite forming water soluble clathrate complexes, the crystalline cyclodextrin drug complexes have been limited in their utility to sublingual administration.

Efforts have been made to inhibit crystal formation in cyclodextrin drug complexes by derivatizing parent

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cyclodextrins in a non-specific manner to obtain amorphous mixtures containing many cyclodextrin derivative components (cf. Pitha; U.S. Patent No. 4,596,795 and 4,727,064). These mixtures prevent the crystallization processes seen
5 with single compounds, providing a lowering of toxicity.

Disclosure of the Invention

The present invention provides purified cyclodextrin derivatives present both as single derivatives and as mixtures of derivatives. These are obtained by heating a
10 cyclodextrin starting material with a reagent(s) which introduces a specific anionic-type substituent, i.e., a (C₂-₆ alkylene)-SO₃⁻anionic substituent, onto the cyclodextrin molecule. These have been discovered to possess notably enhanced aqueous solubility and an advantageously low
15 degree of toxicity. The more highly substituted cyclodextrin derivatives have further been found to advantageously cause essentially no membrane disruption. These derivatized cyclodextrins are useful as clathrating agents in parenteral pharmaceutical formulations and other
20 related uses.

Brief Description of the Figures

A more complete appreciation of this invention and many of its attendant advantages will be readily obtained as the same becomes better understood by the reference to the
25 following detailed description when considered in connection with the accompanying figures, wherein:

FIGURE 1 sets out cumulative urinary cyclodextrin excretion in mice for underivatized cyclodextrin, hydroxy

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propyl-derivatized cyclodextrin, and two sulfoalkyl cyclodextrin derivatives of the present invention;

FIGURES 2 and 3 provide data showing that the more highly substituted alkylsulfonic acids of the present invention
5 cause less membrane disruption, as determined by red blood cell hemolysis studies, as compared to the mono-substituted alkylsulfonic acid derivatives, with the underivatized cyclodextrin causing the most membrane disruption, and that
10 the mono-substituted alkylsulfonic acid derivatives of the present invention cause about the same amount of membrane disruption as does the hydroxypropyl cyclodextrin derivative, as also determined by red blood cell hemolysis study;

FIGURES 4, 5 and 6 show that the association constants
15 for the equilibrium between the sulfoalkyl cyclodextrin derivatives of the present invention and digoxin or progesterone are considerably larger than the association constant for the equilibrium between a hydroxypropyl cyclodextrin derivative and digoxin or progesterone,
20 respectively; and

FIGURES 7, 8, 9 and 10 similarly show that with phenytoin and testosterone the sulfoalkyl cyclodextrin derivatives of the present invention possess notably greater association constants as compared to the hydroxypropyl cyclodextrin
25 derivative.

Best Mode for Carrying Out the Invention

Thus this invention provides cyclodextrin derivatives suitable for pharmaceutical use. These derivatives are suitable for use as clathrating agents with drugs to

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provide clathrate complexes which are useful in parenteral and other pharmaceutical formulations. Procedures for making and isolating the cyclodextrin derivatives are also provided.

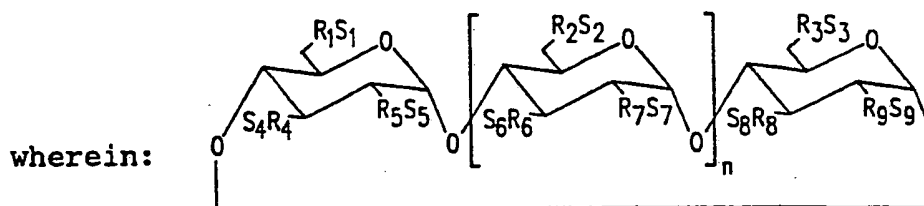
- 5 The cyclodextrin derivatives of the present invention are functionalized with (C₂₋₆ alkylene)-SO₃⁻ groups, and are thus charged species. The fact that these compounds have been discovered to possess a very low level of toxicity is surprising in light of the prior art's belief that
- 10 cyclodextrin derivatives must retain electroneutrality to sustain lack of toxicity (cf. Pitha, "Amorphous Water-Soluble" "Third Int'l Symposium on Recent Advances in Drug Delivery Systems, Salt Lake City, Utah, Feb. 23-27, 1987).
- 15 The high aqueous solubility of the cyclodextrin derivatives of the present invention, and their resulting lowered nephrotoxicity, is further surprising in light of U.S. 4,727,064's disclosure that to maintain a high level of solubility for cyclodextrin derivatives, a mixture of
- 20 derivatives should be used.

The aqueous solubility exhibited by the present sulfoalkyl cyclodextrin derivatives appears to be obtained through solvation of the sulfonic acid moieties. Thus heterogeneous mixture of the present cyclodextrin

25 derivatives is not a requirement for the observed enhanced solvation to occur. Although a mixture of sulfoalkyl ether derivatives can be used in accordance with the present invention, such a mixture is not required for enhanced solubility.

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In a preferred embodiment (1), the cyclodextrin derivatives of this invention have structures represented by formula (2):



5 n is 4, 5 or 6;

$R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8$ and R_9 are each, independently, O^- or a $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group, wherein at least one of R_1 and R_2 is independently a $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group, preferably a $O-(CH_2)_mSO_3^-$ group,
 10 wherein m is 2 to 6, preferably 2 to 4, (e.g. $OCH_2CH_2CH_2SO_3^-$ or $OCH_2CH_2CH_2CH_2SO_3^-$); and

$S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8$ and S_9 are each, independently, a pharmaceutically acceptable cation which includes, for example, H^+ , alkali metals (e.g. Li^+, Na^+ ,
 15 K^+), alkaline earth metals (e.g., Ca^{+2}, Mg^{+2}), ammonium ions and amines cations such as the cations C_{1-6} alkylamines, piperidine, pyrazine, C_{1-6} alkanolamine and C_{4-8} cycloalkanolamine.

In another preferred embodiment (2):

20 R_1 is a $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group, preferably a $O-(CH_2)_mSO_3^-$ group, (e.g. $OCH_2CH_2CH_2SO_3^-$ or $OCH_2CH_2CH_2CH_2SO_3^-$);

R_2 to R_9 are O^- ;

S_1 to S_9 are as defined in embodiment (1) supra.

In another preferred embodiment (3):

R_1 , R_2 and R_3 are each, independently, a $O-(C_{2-6}\text{-alkylene})-SO_3^-$ group, preferably a $O-(CH_2)_mSO_3^-$ group, (e.g. $OCH_2CH_2CH_2SO_3^-$ or $OCH_2CH_2CH_2CH_2SO_3^-$);

5 R_4 to R_9 are O^- ; and

S_1 to S_9 are as defined in embodiment (1) supra.

In another preferred embodiment (4):

R_1 to R_3 are as defined in embodiments (2) or (3); supra;

at least one of R_4 , R_6 and R_8 is a $O-C_{2-6}\text{-alkylene})-SO_3^-$
10 group, preferably a $O-(CH_2)_m-SO_3^-$ group (e.g. $OCH_2CH_2CH_2SO_3^-$
or $OCH_2CH_2CH_2CH_2SO_3^-$).

R_5 , R_7 and R_9 are O^- ; and

S_1 to S_9 are as defined in embodiment (1) supra.

In another preferred embodiment (6):

15 R_1 , R_2 , R_3 , R_4 , R_6 and R_8 are each, independently, a
 $O-(C_{2-6}\text{-alkylene})-SO_3^-$ group, preferably a $O-(CH_2)_mSO_3^-$
group (e.g. $OCH_2CH_2CH_2SO_3^-$ or $OCH_2CH_2CH_2CH_2SO_3^-$);

R_5 , R_7 and R_9 are O^- ; and

S_1 to S_9 are as defined in embodiment (1) supra.

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The terms "alkylene" and "alkyl" in this text (e.g., in the $O-(C_{2-6}\text{-alkylene})SO_3^-$ group or in the alkylamines) include both linear and branched, saturated and unsaturated (i.e., containing one double bond) divalent alkylene groups 5 and monovalent alkyl groups, respectively. The term "alkanol" in this text likewise includes both linear and branched, saturated and unsaturated alkyl components of the alkanol groups, in which the hydroxyl groups may be situated at any position on the alkyl moiety. The term 10 "cycloalkanol" includes unsubstituted or substituted (e.g., by methyl or ethyl) cyclic alcohols.

The present invention provides compositions containing a mixture of cyclodextrin derivatives having the structure set out in formula (2), where the composition overall 15 contains on the average at least 1 and up to $3n + 6$ alkylsulfonic acid moieties per cyclodextrin molecule. The present invention also provides compositions containing essentially only one single type of cyclodextrin derivative.

20 The present cyclodextrin derivatives are either substituted at least at one of the primary hydroxyl group (i.e., at least one of R_1 to R_3 is a substituent), or they are substituted at both the primary hydroxyl group and at the 3-position hydroxyl group (i.e., both at least one of 25 R_1 to R_3 and at least one of R_4 , R_6 and R_8 are a substituent). Substitution at the 2-position hydroxyl group, while theoretically possible, on the basis of the inventors' studies, does not appear to appear to be substantial in the products of the invention.

30 The cyclodextrin derivatives of the present invention are obtained (as discussed below) as purified compositions,

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i.e., compositions containing at least 95 wt.% of cyclodextrin derivative(s) with the substitution occurring at least on the primary hydroxyl group of the cyclodextrin molecule (i.e. R_1 , R_2 or R_3 of formula (2)), as determined by 300 MHz ^1H NMR). In a preferred embodiment, purified compositions containing at least 98 wt.% cyclodextrin derivative(s) can be obtained.

This is to be contrasted with the U.S. 3,426,011 disclosure which reports obtaining only reaction products of the reaction of a cyclodextrin with a sultone reactant. These reaction products contain considerable quantities of unsubstituted cyclodextrin starting material.

In all of the compositions of the invention unreacted cyclodextrin has been substantially removed, with the remaining impurities (i.e., ≤ 5 wt.% of composition) being inconsequential to the performance of the cyclodextrin derivative-containing composition.

The more highly substituted alkyl sulfonic acid cyclodextrin derivatives of the present invention have been discovered to possess, in addition to notably enhance solubility characteristics and low toxicity, the advantageous property of causing less membrane disruption. In red blood cell hemolysis studies, the more highly substituted cyclodextrin derivatives demonstrated negligible membrane disruption. The mono-substituted cyclodextrin derivatives caused about the same amount of membrane disruption as the hydroxy propyl derivative.

Preparation of the Cyclodextrin (CD) Derivatives:

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The cyclodextrin derivatives described may be generally prepared by dissolving the cyclodextrin in aqueous base at an appropriate temperature, e.g., 70° to 80°C, at the highest concentration possible. For example, to prepare 5 the cyclodextrin derivatives of embodiment (4), an amount of an appropriate alkyl sultone, corresponding to the number of moles of primary CD hydroxyl group present, is added with vigorous stirring to ensure maximal contact of the heterogeneous phase.

- 10 To prepare the cyclodextrin derivatives of the embodiment (2) a molar amount of the alkyl sultone, corresponding to the number of moles of CD used, is used. As would be readily determinable by one of skill in this art, to prepare cyclodextrin derivatives of embodiment (1), which 15 encompasses both cyclodextrin derivatives embodiments (4) and (2), an amount of alkyl sultone between that stated above is used. Other cyclodextrin derivatives provided by the present invention are prepared Mutatis Mutandis.

The mixtures are allowed to react until one phase results 20 which is indicative of depletion of the alkyl sultone. The reaction mixture is diluted with an equal volume of water and neutralized with an acid such as hydrochloric acid. The solution is then dialyzed to remove impurities followed by concentration of the solution by ultrafiltration.

- 25 The concentrated solution is then subjected to ion-exchange chromatography to remove unreacted cyclodextrin, and then freeze-dried to yield the desired product.

The CD used in this invention may be any CD obtained by 30 known methods, e.g., by the action of cyclodextrin-

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glucanotransferase (CGTase, E.C., 2.4.1.19.) upon starch. Thus CD herein means α -CD in which six glucose units are linked together through α -1,4 bond, β -CD in which seven glucose units are linked together, or γ -CD in which eight glucose units are linked together, or a mixture thereof. Of these, use of β -CD is most preferred for production of partially derivatized products of broad utility.

As noted above and depending on the cyclodextrin derivative sought, the amount of alkyl sultone used as the derivatizing agent should be not more than about one molar equivalent, based on the number of primary hydroxyl groups present in the CD, although the optimum amount may be somewhat dependent on the reactant concentration. Lithium hydroxide, sodium hydroxide and potassium hydroxide may be used as the accelerator. Of these, sodium hydroxide is preferable because of its low cost. Its amount must be more than about 30 molar equivalents, and should preferably be in the range of 80 to 200 molar equivalents, with the reactant concentration being set at a level higher than 10% (wt/wt), preferably in the range of 40 to 60% (wt/wt).

Any solvent which is substantially inert to the partial alkylation may be used as reaction medium. Typical examples are water, DMF, DMSO, and mixtures thereof, but use of water alone is most preferred for ease of after-treatment.

The type and concentration of alkylsultone and alkali are not critical to the reaction. However, the reaction is normally carried out with stirring at 10 to 80°C for one hour, preferably at 20° to 50°C for 5 to 20 hours.

Techniques commonly used in this field may be employed to isolate and purify the objective compounds from reaction

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mixtures. These include extraction with organic solvents, dialysis, adsorption chromatography with activated charcoal, silica gel, alumina and other adsorbents, chromatography using, as carrier, crosslinked dextrin, 5 styrene/divinylbenzene copolymers and other cross-linked polymers, and combinations thereof.

Preparation of the Clathrate Complexes:

The clathrate complexes of the invention may be prepared by any method known in the art for the preparation of 10 complexes of cyclodextrins.

For example, to prepare the clathrate complexes, a cyclodextrin derivative dissolved in water or in an organic solvent miscible with water may be added to a physiologically active compound (drug) dissolved in an 15 organic solvent which is miscible with water. After the mixture is heated, the desired product is obtained by concentrating the mixture under reduced pressure or leaving it to be cooled. In this case, the mixing ratio of organic solvent with water may be suitably varied according to the 20 solubilities of the starting materials and products.

Examples of drugs which may be complexed with the cyclodextrin derivatives include diphenyl hydantoin, adiphenine, allobarbitol, aminobenzoic acid, amobarbital, ampicillin, anethole, aspirin, azopropazone, azulene 25 barbituric acid, beclomethasone, beclomethasone dipropionate, bencyclane, benzaldehyde, benzocaine, benzodiazepines, benzothiazide, betamethasone, betamethasone 17-valerate, bromobenzoic acid, bromoisovalerylurea, butyl-p-aminobenzoate,

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- chloralhydrate, chlorambucil, chloramphenicol,
chlorobenzoic acid, chlorpromazine, cinnamic acid,
clofibrate, coenzyme A, cortisone, cortisone acetate,
cyclobarbitol, cyclohexyl anthranilate, deoxycholic acid,
5 dexamethasone, dexamethasone acetate, diazepam, digitoxon,
digoxin, estradiol, flufenamic acid, fluocinolone
acetone, 5-fluorouracil, flurbiprofen,
griseofulvin, guaiazulene, hydrocortisone, hydrocortisone
acetate, ibuprofen, indican, indomethacin, iodine,
10 ketoprofen, lankacidin-group antibiotics, mefanamic acid,
menadione, mephobarbital, methbarbital, methicillin,
metronidazole, mitomycin, nitrazepam' nitroglycerin'
nitrosureas, paramethasone, penicillin, pentobarbital,
phenobarbital,
15 phenobarbitone, phenyl-butyric acid, phenyl-valeric acid,
phenytoin, prednisolone, prednisolone acetate,
progesterone, propylparaben, proscillaridin, prostaglandin
A series, prostaglandin B series, prostaglandin E series,
prostaglandin F series, quinolone anti microbiacs,
20 reserpine, spironolactone, sulfacetamide sodium,
sulphonamide, testosterone, thalidomide, thiamine
dilaurylsulphate, thiamphenicolpalmitate, thiopental,
triamcinolone, vitamin A, vitamin D3, vitamin E, vitamin K3
and warfarin.
- 25 The drug may be dissolved in water or an organic solvent
(either miscible or immiscible with water). Convenient
solvents include for example diethylether, tetrahydrofuran,
dioxane, acetone, dimethylsulfoxide, dimethylformamide and
lower aliphatic alcohols. Preferably the drug is dissolved
30 in either water or a mixture of water and a water-miscible
solvent such as methanol or ethanol. The drug may also be
suspended in water.

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After equilibrium is reached, the complex may be isolated by any suitable technique for example lyophilization, evaporation of the solvent, precipitation, low temperature crystallization, or spray-drying. Cyclodextrin inclusion
5 complexes may also be produced by physically grinding or kneading the cyclodextrin and the guest molecule with or without a small amount of solvent.

The ratio of cyclodextrin derivative to drug used to prepare the clathrate complexes of the invention may be any
10 convenient ratio but conveniently the cyclodextrin derivative is used in a molar excess.

The benefits derived from the invention may be obtained by having the molar ratio of cyclodextrin derivative to drug in the range of 10:1 to 1:10 preferably 2:1 to 5:1 for
15 example 3:1 and by using the methods and ratios described above. Complexes are conveniently obtained containing up to 20% w/w of the drug. However in view of the low doses of the drug normally administered and the difficulty of preparing homogenous mixtures of active ingredient and
20 excipients it may be desirable to prepare the complex with an excess of the cyclodextrin derivative present, for example complexes containing in the order of 0.1 to 10% by weight of the drug, particularly in the range 0.5 to 0.2% by weight.

25 The clathrate complexes of the invention provide a more convenient way of administering the drugs, the cyclodextrin acting merely as a solubilizing agent without altering the therapeutic behavior of the drug in any way.

Composition Containing the Clathrate Complexes of the
30 Invention:

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The invention thus also provides an inclusion complex as defined herein for use in human or veterinary medicine. The complex, for use as a pharmaceutical, may be presented as a pharmaceutical formulation.

5 The invention therefore provides in a further aspect a pharmaceutical formulation comprising an inclusion complex of a drug with a cyclodextrin derivative together with a pharmaceutically acceptable carrier therefor and optionally other therapeutic and/or prophylactic ingredients. The
10 carriers must be "acceptable" in the sense of being compatible with the other ingredients of the formula and not deleterious to the recipient thereof. Suitably the pharmaceutical formulation will be in unit dosage form. Each unit dose will conveniently contain that amount of
15 drug normally incorporated into a unit dose of such drug in the absence of a cyclodextrin.

The pharmaceutical formulations may be any formulation in which the inclusion complexes may be administered and include those suitable for oral, intranasal, intraocular or
20 parenteral (including intramuscular and intravenous) administration. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into
25 association the active compound with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral
30 administration wherein the carrier is a solid are most preferably presented as unit dose formulations such as

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boluses, capsules, cachets or tablets each containing a predetermined amount of the active ingredient. A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding an inert liquid diluent. Tablets may be optionally coated and, if uncoated, may optionally be scored. Capsules may be prepared by filling the active compound, either alone or in admixture with one or more accessory ingredients, into the capsule cases and then sealing them in the usual manner. Cachets are analogous to capsules wherein the active ingredient together with any accessory ingredient(s) is sealed in a rice paper envelope.

Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for manufacture of tablets. These excipients may be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium, phosphate or sodium phosphate; granulating and disintegrating agents, for example maize starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

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The present invention also provides the complexes of the present invention in pharmaceutical formulations exhibiting sustained release of a drug. Pharmaceutical formulations exhibiting sustained release of a drug are generally known. 5 Such formulations include devices made of inert polymers or biodegradable poly-acids in which the active ingredient (the present complex) is either dispersed, covalently linked via labile bonds, or stored as a reservoir between polymer membranes. Sustained release is achieved through 10 diffusion of the active ingredient through the polymer matrix or hydrolysis of any covalent linkages present.

Sustained release may also be presented by delivery of the active ingredient via osmotic pumps. Osmotic pumps consist of a reservoir of solution or suspension of active 15 ingredient (i.e., the present complex) surrounded by a semipermeable membrane containing a drug portal. As water penetrates through the semipermeable membrane into the complex reservoir, the complex solution is pushed through the portal and released.

20 The cyclodextrin derivatives of the invention act as drug solubilizing agents in these systems. The present cyclodextrin derivatives can also act as osmotic driving agents providing potential for the influx of water in such systems.

25 Pharmaceutical formulations suitable for oral administration wherein the carrier is liquid may conveniently be presented as a solution in an aqueous liquid or a non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion. Pharmaceutical formulations 30 suitable for parenteral administration are conveniently presented in unit dose or multi-dose containers which are

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sealed after introduction of the formulation unit required for use.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed
5 with an inert solid diluent, for example calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example arachis oil, peanut oil, liquid paraffin or olive oil.

10 It should be understood that in addition to the aforementioned carrier ingredients, the pharmaceutical formulations described above may include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface
15 active agents, thickeners, lubricants, preservatives (including anti-oxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient.

For these purposes the compounds of the present invention
20 may be administered orally, topically, intranasally, intraocularly, parenterally, by inhalation spray or rectally in dosage unit formulations containing conventional non-toxic
a pharmaceutically acceptable carriers, adjuvants and
25 vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition to the treatment of warm-blooded animals such as mice, rats, horses, dogs, cats, etc., the compounds of the
30 invention are effective in the treatment of humans.

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Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, 5 methylcellulose, hydroxypropylmethylcellulose sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty 10 acids, for example of polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids 15 and a hexitol such as polyoxyethylene sorbitol mono-oleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan mono-oleate. The aqueous suspensions may also contain one or more preservatives, for 20 example, ethyl or n-propyl p-hydroxy benzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis 25 oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oil suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to 30 provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

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Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more
5 preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

10 The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oils, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be
15 naturally-occurring gums, for example gum acacia or gum tragacanth, naturally occurring phosphatides, for example soya bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan mono-oleate, and condensation products of the said
20 partial esters with ethylene oxide, for example polyoxyethylene sorbitan mono-oleate. The emulsions may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, sorbitol or sucrose. Such
25 formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated
30 according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may

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also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, jellies, solutions or suspensions, etc. containing the active ingredient are employed.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may contain from 1.0 to 750 mg. of active agent compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 weight percent of the total composition. Unit dosage forms will generally

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contain between from about 1 to about 500 mg. of active ingredient.

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Administration of the Clathrate Complexes to a Patient:

It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

Pharmaceutical formulations containing inclusion complexes may be administered at dosage levels and dosage intervals required to achieve the desired pharmacologic response normally associated with the drug and the disease state in absence of the cyclodextrin.

The other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

Examples

The hydroxypropyl cyclodextrin derivative used in the experiments reported below was purchased from Pharmatec, Inc., Alachua, Fl.

Preparation of the cyclodextrin derivative of the invention:**Example 1: Mono-sulfobutyl ether of β -cyclodextrin**

In a 100 mL round-bottom flask, 10 grams β -cyclodextrin (8.81×10^{-3} mole) was added with stirring to an aqueous

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solution composed of 30 mL water and 5.0 grams sodium hydroxide maintained at 70°C. To this solution 1.8 mL (2.40 gm, 1.76×10^{-2} mole) butane sultone was slowly added with vigorous stirring to insure maximal contact of the 5 heterogenous phases.

After a single phase was observed indicating depletion of the alkyl sultone, the solution was cooled to room temperature and diluted with 20 mL water. The resulting solution was neutralized with 1 N hydrochloric acid and 10 dialyzed against 3 x 700 mL water to remove salts and hydroxyalkyl sulfonic acids formed as side products.

The diasylate was concentrated by ultrafiltration and placed on an ion-exchange column composed of 50 grams A-25 DEAE-Sephadex packed in a 1.25 inch i.d. glass column. 15 Unreacted β -cyclodextrin was removed by elution with distilled water. The mono-substituted sulfobutyl ether of β -cyclodextrin was isolated by elution with 0.1 N sodium hydroxide. The effluent fraction containing the monosubstituted derivative was ultrafiltered to remove any 20 residual salts. The pH of the retentate was adjusted to neutrality and lyophilized to obtain 2.17 grams of the monosubstituted sulfobutyl ether of β -cyclodextrin as a white amorphous solid. Elemental analysis of the product showed a carbon to sulfur ratio of 13.7 which corresponds 25 to Ca. 1.2 substitutions per molecule.

Example 2: Mono-sulfopropyl ether of β -cyclodextrin

The processes of Example 1 were repeated substituting 1.54 mL (2.15 grams, 1.76×10^{-2} mole) propane sultone for butane sultone to yield 1.97 grams mono-sulfobutyl ether of 30 β -cyclodextrin as a white amorphous solid. Elemental

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analysis of the product showed a carbon to sulfur ratio of 12.1 which corresponds to Ca. 1.4 substitutions per molecule.

Example 3 : Sulfobutyl ethers of β -cyclodextrin

5 In a 50 mL round-bottom flask, 5 grams β -cyclodextrin
(4.41×10^{-3} mole) was added with stirring to an aqueous
solution composed of 10 mL water and 2.0 grams sodium
hydroxide maintained at 70°C. To this solution 4.5 mL (6.0
gm, 4.41×10^{-2} mole) butane sultone was slowly added with
10 vigorous stirring to insure maximal contact of the
heterogenous phases. After a single phase was observed
indicating depletion of the alkyl sultone, the solution was
cooled to room temperature and diluted with 20 mL water.
The resulting solution was neutralized with 1 N
15 hydrochloric acid and dialyzed against 3 x 700 mL water to
remove salts and hydroxyalkyl sulfonic acids formed as side
products. The diasylate was concentrated by
ultrafiltration and the pH of the retentate was adjusted to
neutrality and lyophilized to obtain the sulfobutyl ether
20 of β -cyclodextrin as a white amorphous solid. Elemental
analysis of the product showed a carbon to sulfur ratio of
3.73 which corresponds to Ca. 7 substitutions per molecule.
Thin-layer chromatography of the product (2-
butanone:methanol:water, 4:4:2) shows the absence of
25 unreacted β -cyclodextrin.

Example 4: Additional sulfoalkylethers of cyclodextrin

The processes of Example 3 were varied with regard to
reactants and molar ratios to obtain cyclodextrin
derivatives with varying degrees of substitution.
30 Representative results follow:

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	<u>Cyclodextrin</u>	<u>Moles</u>	<u>Sulton</u>	<u>Moles</u>	<u>Carbon/Sulfur Ratio</u>	<u>Substitutions</u>
	β -.....	4.41×10^{-3}	propane....	4.4×10^{-2}	3.28.....	7.2
	β -.....	4.41×10^{-3}	propane....	2.2×10^{-2}	5.53.....	3.6
	β -.....	4.41×10^{-3}	butane.....	2.2×10^{-2}	4.83.....	4.7
5	γ	1.54×10^{-3}	propane....	7.71×10^{-3}	*.....	3.5
	γ -.....	1.54×10^{-3}	butane.....	7.71×10^{-3}	*.....	3.2

* Substitution determined by peak areas from $^1\text{H-NMR}$ spectra.

Cumulative Urinary Cyclodextrin Excretion:

The data provided in the group set out in Figure 1 indicates that although the sulfoalkyl cyclodextrin derivatives of the invention as well as the hydroxypropyl derivative are excreted faster and to a greater extent in mice than the parent compound, the present derivatives are excreted fastest. Figure 1 provides data for the underivatized cyclodextrin compound, the hydroxypropyl derivative, the sulfobutyl derivative of the invention, and the sulfopropyl derivative of the invention.

Acute Parenteral Toxicity

The sulfoalkyl cyclodextrin derivatives of the invention exhibited no observable toxic effects in male mice over a 30 day period following intraperitoneal of 5.49×10^{-3} mol/Kg.

This dose is equivalent to 7.1 gm/Kg for the monosulfoalkyl derivatives, 12.3 gm/Kg for the sulfobutyl derivative w/7 degrees of substitution, and 11.8 gm/Kg for the sulfopropyl derivative w/7 degrees of substitution.

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Plasma Urea Nitrogen:

Plasma urea nitrogen levels are an indicator of kidney function with higher levels indicating renal damage. The data in Table 1 indicates that the sulfoalkyl cyclodextrin derivatives of the invention do not cause increased plasma urea nitrogen levels in mice as compared to the underivatized parent compound (control). There is however no statistical difference between our derivatives and the hydroxypropyl derivative.

10 **Table 1****Plasma Urea Nitrogen⁽¹⁾**

	<u>Sample time (hrs)</u>	<u>PUN\pmS.D. (mg/dL)⁽²⁾</u>
Control (normal saline)	24	15.88 \pm 1.21
β -Cyclodextrin	24	160.10 \pm 26.16
15 Moleculon TM (hydroxypropyl derivative)	24	15.43 \pm 1.50
Sulfopropyl ether of β -Cyclodextrin (3.6 substitution per CD molecule)	24	15.27 \pm 0.71
Sulfobutyl ether of β -Cyclodextrin (4.7 substitution per CD molecule)	24	14.42 \pm 0.46

20 ⁽¹⁾ SIGMA Urea Nitrogen Procedure No. 640-A⁽²⁾ n=4**Hemolysis of Red Blood Cells:**

As can be seen from the data in Figures 2 and 3 the more highly substituted alkylsulfonic acid derivatives of the invention caused less membrane disruption as indicated by the percent hemolysis than the mono substituted derivatives. The mono substituted derivatives caused about the same amount of membrane disruption as the hydroxypropyl derivative.

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Phase Solubility Behavior:

As can be seen from Table 2 below and the data provided in Figures 4a and 4b the association constants for the equilibrium between the sulfobutyl derivatives of the invention and Digoxin are 5 times larger than that of the hydroxypropyl derivative.

Table 2DIGOXIN ASSOCIATION CONSTANTS

		$K_{1:1}(M^{-1})$
10	β -Cyclodextrin	2.82×10^4
	Moleculsol™ (hydroxylpropyl- β -CD)	4.90×10^3
	Sulfopropyl ether of β -Cyclodextrin	
	(1 sub) ⁽¹⁾	2.76×10^4
	(4.8 subs)	1.71×10^4
15	(7 subs)	6.88×10^3
	Sulfobutyl ether of β -Cyclodextrin	
	(1 sub)	2.74×10^4
	(3.6 subs)	1.41×10^4
	(7 subs)	5.29×10^3

20 ⁽¹⁾ No. of substituents per CD molecule

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Table 3

<u>ASSOCIATION CONSTANTS PROGESTERONE</u>		
		$k_{1:1}(m^{-1})$
	β -Cyclodextrin	-
5	Molecusol [®] (hydroxypropyl β -CD)	1.12×10^4
	<u>Sulfobutyl ether of β-Cyclodextrin</u>	
	(1 sub) ⁽¹⁾	1.72×10^4
	(4.7 subs)	1.57×10^4
	(7 subs)	1.83×10^4
10	<u>Sulfopropyl ether of β-Cyclodextrin</u>	
	(1 sub)	1.66×10^4
	(3.6 subs)	1.19×10^4
	(7 subs)	7.68×10^3
<hr/>		
⁽¹⁾ No. of substituents per CD molecule		
<hr/>		

15 It should be noted that the x axis for the graphs of
 Figures 4a and 4b have a maximum of ~1.8% w/w cyclodextrin.
 If the relative solubilizing ability of the present
 derivatives is considered relative to that for the
 hydroxypropyl derivative (at 50% solutions as is done in
 20 U.S. 4,727,064, Table 1) the apparent solubility of digoxin
 is ~216 mg/ml for the present sulfobutyl derivatives as
 compared to ~80 mg/ml for the hydroxypropyl derivative.
 The value of 45.0 mg/mL reported in U.S. 4,727,064 was for
 a hydroxypropyl derivative with a different degree of
 25 substitution than the hydroxypropyl derivative used herein
 for comparison.

Similar results can be seen for progesterone (see Table 3
 and Figures 5 and 6), phenytoin (see Table 4 and Figures 7
 and 8), and testosterone (see Table and Figures 9 and 10).

Table 5**TESTOSTERONE ASSOCIATION CONSTANTS**

		$k_{1:1}(m^{-1})$
5	β -Cyclodextrin	1.78×10^4
	Moleculsol [™] (hydroxylpropyl β -CD)	1.16×10^4
	(1 sub) (1)	1.64×10^4
	Sulfobutyl ether β -Cyclodextrin	
	(4.7 subs)	1.82×10^4
10	(7 subs)	2.25×10^4
	Sulfopropyl ether β -Cyclodextrin	
	(1 sub)	1.87×10^4
	(3.6 subs)	1.43×10^4
	(7 subs)	9.63×10^3

(1) No. of substituents per CD molecule

Table 4**PHENYTOIN ASSOCIATION CONSTANTS**

		$k_{1:1}(m^{-1})$
20	β -Cyclodextrin	1.51×10^3
	Moleculsol [™] (hydroxylpropyl β -CD)	1.07×10^3
	(1 sub) (1)	1.22×10^3
	Sulfobutyl ether β -Cyclodextrin	
	(4.7 subs)	1.26×10^3
25	(7 subs)	7.56×10^3
	Sulfopropyl ether β -Cyclodextrin	1.03×10^3
	(1 sub)	
	(3.6 subs)	1.31×10^3
	(7 subs)	8.24×10^2

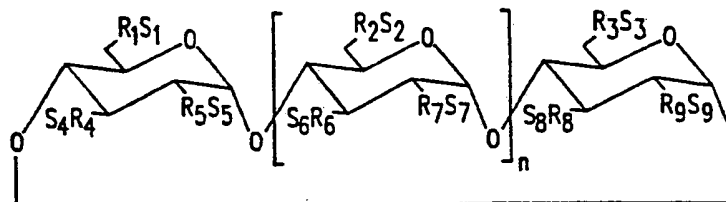
(1) No. of substituents per CD molecule

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Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be
5 practiced otherwise than as specifically described herein.

CLAIMS

1. A purified cyclodextrin derivative of formula (2):



wherein:

- 5 n is 4, 5 or 6;

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 and R_9 are each, independently, O^- or a $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group, and at least one of R_1 and R_2 is, independently, said $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group, and

- 10 S_1 , S_2 , S_3 , S_4 , S_5 , S_6 , S_7 , S_8 and S_9 are each, independently, a pharmaceutically acceptable cation.

2. The derivative of Claim 1, wherein R_1 , R_2 and R_3 are each, independently, $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group.

3. The derivative of Claim 1, wherein at least one of R_1 ,
15 R_2 and R_3 are each, independently, $O-(CH_2)_m-SO_3^-$ group, wherein m is 2, 3, 4, 5 or 6.

4. The derivative of Claim 1, wherein R_1 , R_2 and R_3 are each, independently, $O-(CH_2)_m-SO_3^-$ group, wherein m is 2, 3, 4, 5 or 6.

5. The derivative of Claim 1, wherein:

at least one of R_4 , R_6 and R_8 is, independently, said $O-(C_{2-6}\text{-alkylene})-SO_3^-$ group; and

R_5 , R_7 and R_9 are all O^- .

5 6. The derivative of Claim 2, wherein:

at least one of R_4 , R_6 and R_8 is, independently, said $O-(C_{2-6}\text{-alkylene})-SO_3^-$ group; and

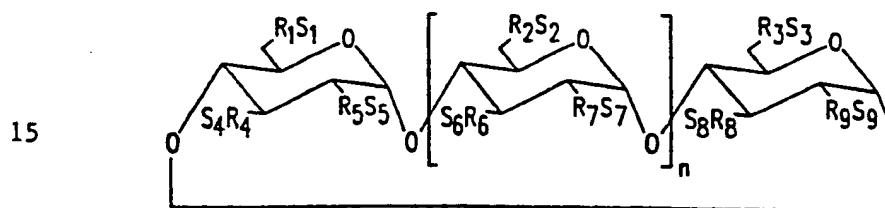
R_5 , R_7 and R_9 are all O^- .

7. The derivative of Claim 2, wherein:

10 R_4 , R_6 and R_8 is, independently, said $O-(C_{2-6}\text{-alkylene})-SO_3^-$ group; and

R_5 , R_7 and R_9 are all O^- .

8. A composition comprising a drug complexed to a cyclodextrin derivative of formula (2):



wherein:

$n = 4, 5 \text{ or } 6;$

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$R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8$ and R_9 are each, independently, O^- or a $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group, and at least one of R_1 and R_2 is, independently, said $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group; and

- 5 S_1 to S_9 are each, independently, a pharmaceutically acceptable cation;

wherein said composition contains not more than 5 wt.% of underivatized cyclodextrin.

9. The composition of Claim 8, wherein R_1, R_2 and R_3 are
10 each, independently, said $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group.

10. The composition of Claim 8, wherein:

at least one of R_4, R_6 and R_8 is, independently, said $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group; and

R_5, R_7 and R_9 are all O^- .

- 15 11. The composition of Claim 9, wherein:

at least one of R_4, R_6 and R_8 is, independently, said $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group; and

R_5, R_7 and R_9 are all O^- .

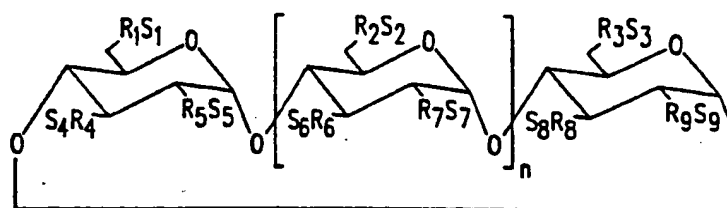
12. The composition of Claim 11, wherein R_4, R_6 and R_8 are
20 each, independently, said $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group.

13. The composition of Claim 8, wherein said drug is one member selected from the group consisting of but not limited to amobarbital, aspirin, beclomethasone,

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benzocaine, benzodiazepines, betamethasone, chlorambucil, chloramphenicol, chlorpromazine, clofibrate, coenzyme A, cortisone, cortisone acetate, cyclobarbitol, dexamethasone, dexamethasone acetate, diazepam, digitoxon, digoxin,
 5 estradiol, 5-fluorouracil, flurbiprofen, griseofulvin, hydrocortisone, hydrocortisone acetate, ibuprofen, indomethacin, ketoprofen, methicillin, metronidazole, mitomycin, nitrazepam, nitroglycerin, penicillin, pentobarbital, phenopbarbital, phenobarbitone, phenyltoin,
 10 prednisolone, prednisolone acetate, progesterone, prostaglandin A series, prostaglandin B series, prostaglandin E series, prostaglandin F series, reserpine, sulfacetamide sodium, testosterone, vitamin A, vitamin D3, vitamin E, vitamin K3, and warfarin.

15 14. A pharmaceutically acceptable composition comprising a pharmaceutically acceptable carrier and a clathrate complex comprising a drug complexed to a cyclodextrin derivative of formula (2):



20 wherein:

$n = 4, 5 \text{ or } 6;$

$R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8$ and R_9 are each, independently, O^- or a $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group, and at least one of R_1 and R_2 is, independently, said $O-(C_{2-6}$
 25 alkylene)- SO_3^- group; and

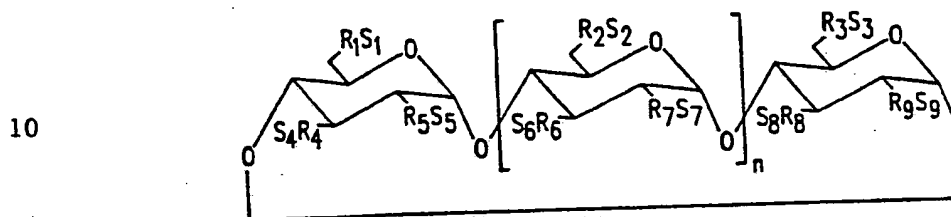
-38-

S_1 to S_9 are each, independently, a pharmaceutically acceptable cation;

wherein said composition contains not more than 5 wt.% of underivatized cyclodextrin.

5 15. The composition of Claim 14, wherein said carrier is a parenterally suitable carrier.

16. A delayed release pharmaceutical composition, comprising a drug complexed to a purified cyclodextrin derivative of formula (2):



wherein:

$n = 4, 5$ or 6 ;

$R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8$ and R_9 are each, independently, O^- or a $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group, and at
 15 least one of R_1 and R_2 is, independently, said $O-(C_{2-6} \text{ alkylene})-SO_3^-$ group; and

S_1 to S_9 are each, independently, a pharmaceutically acceptable cation.

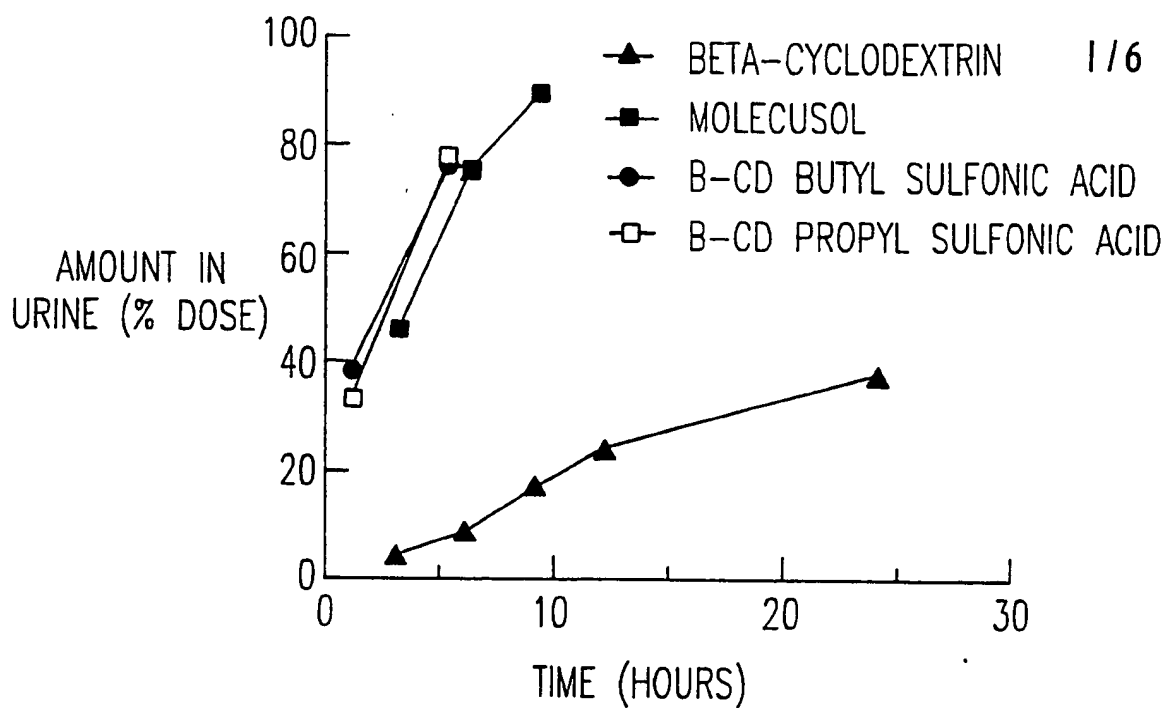


FIG. 1

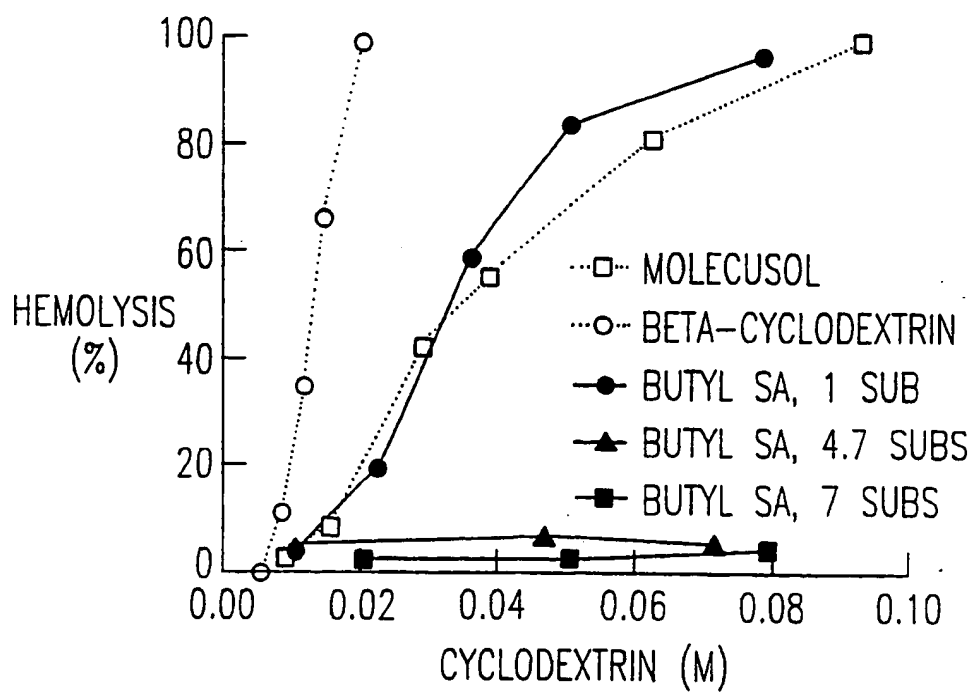
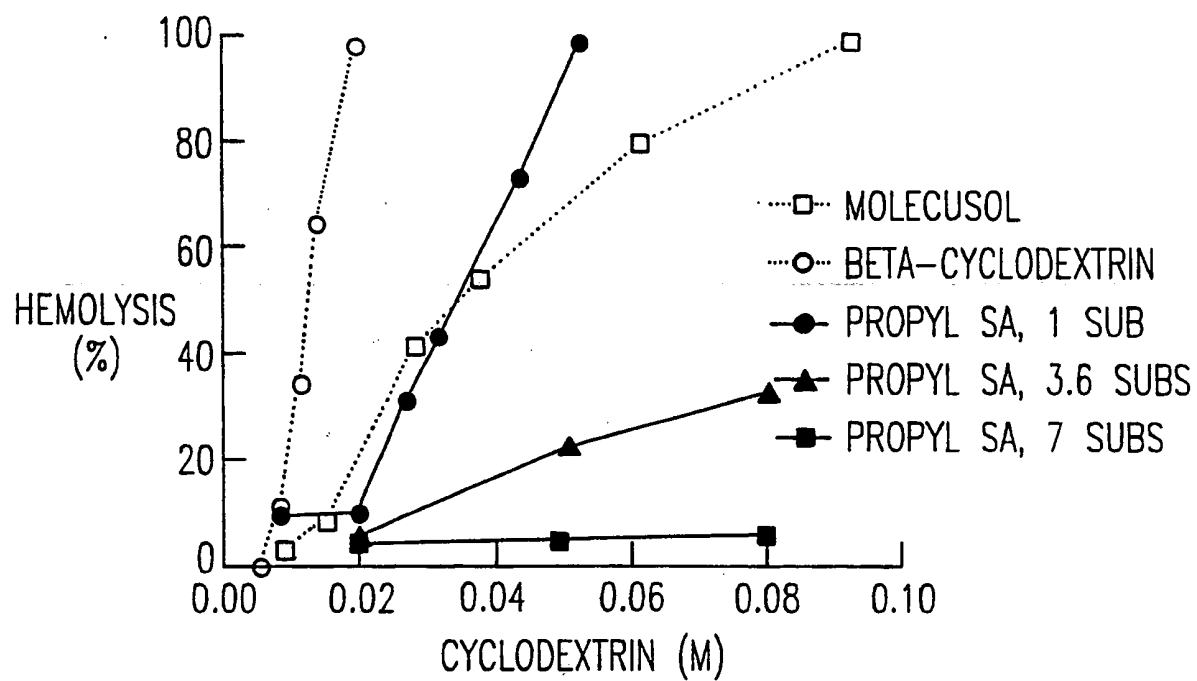
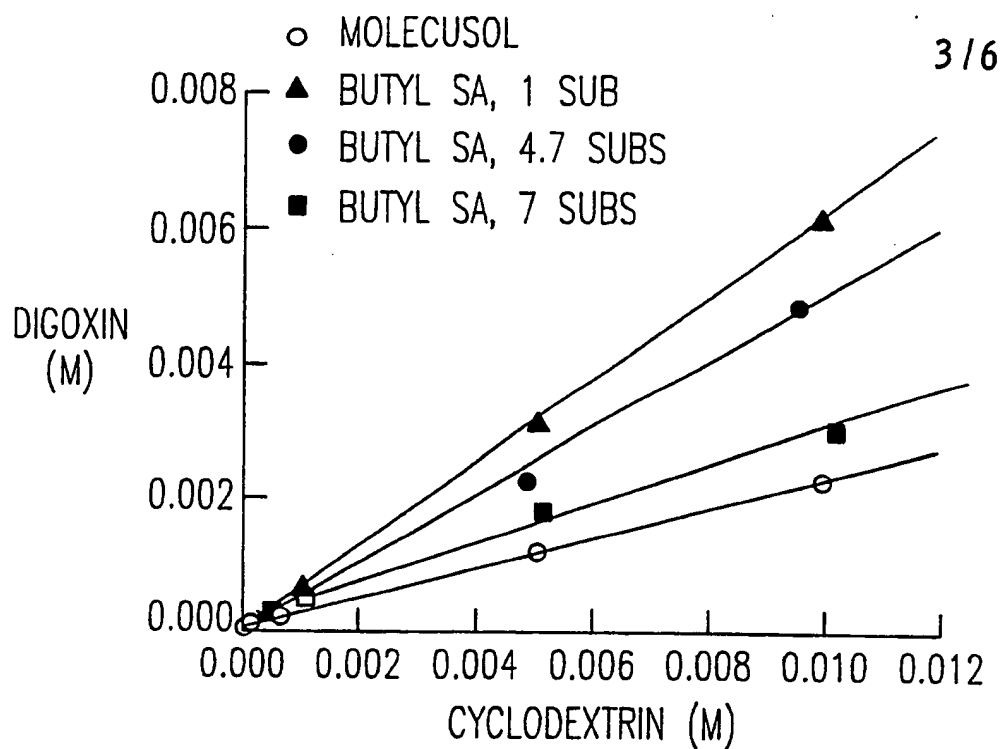
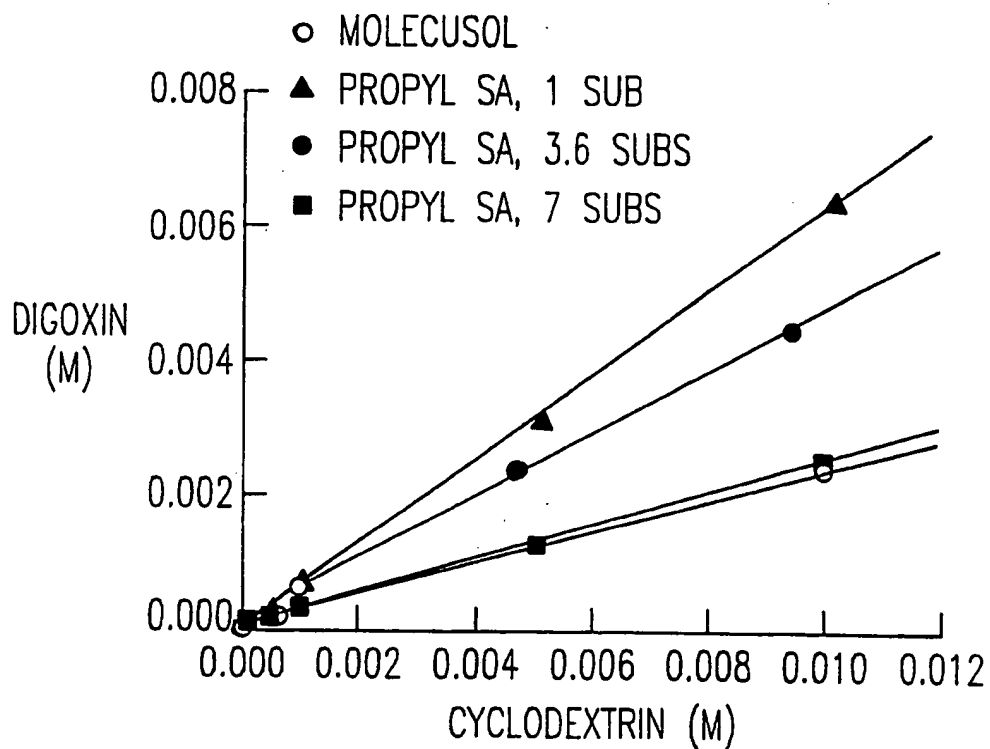
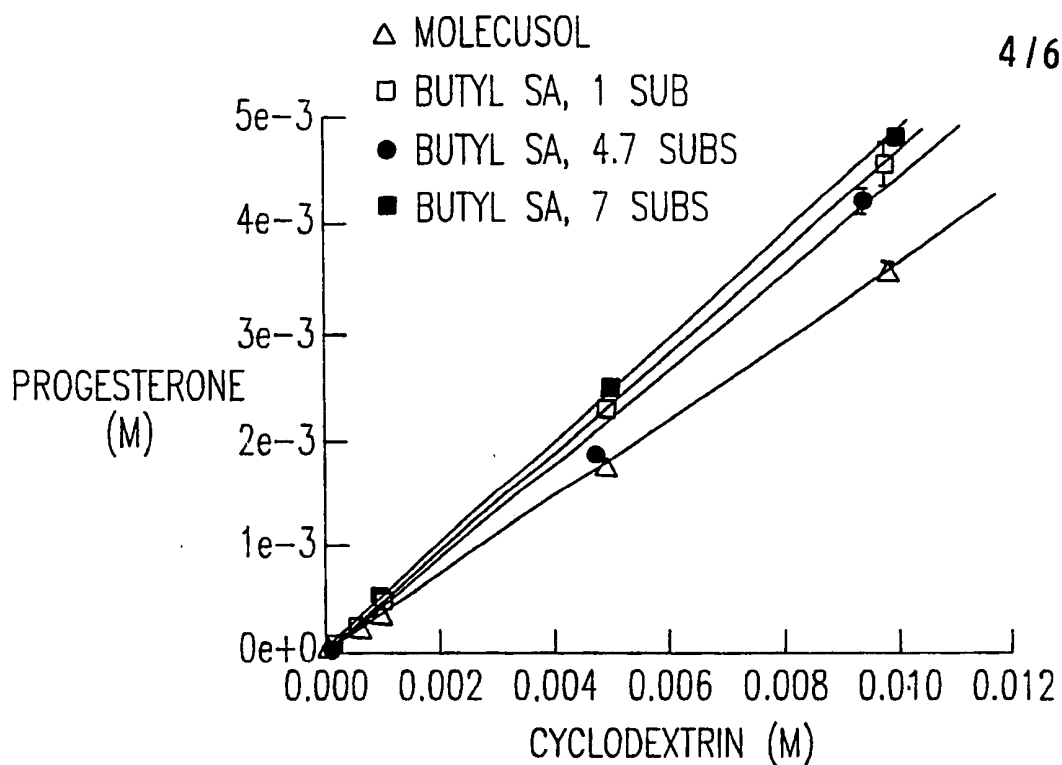
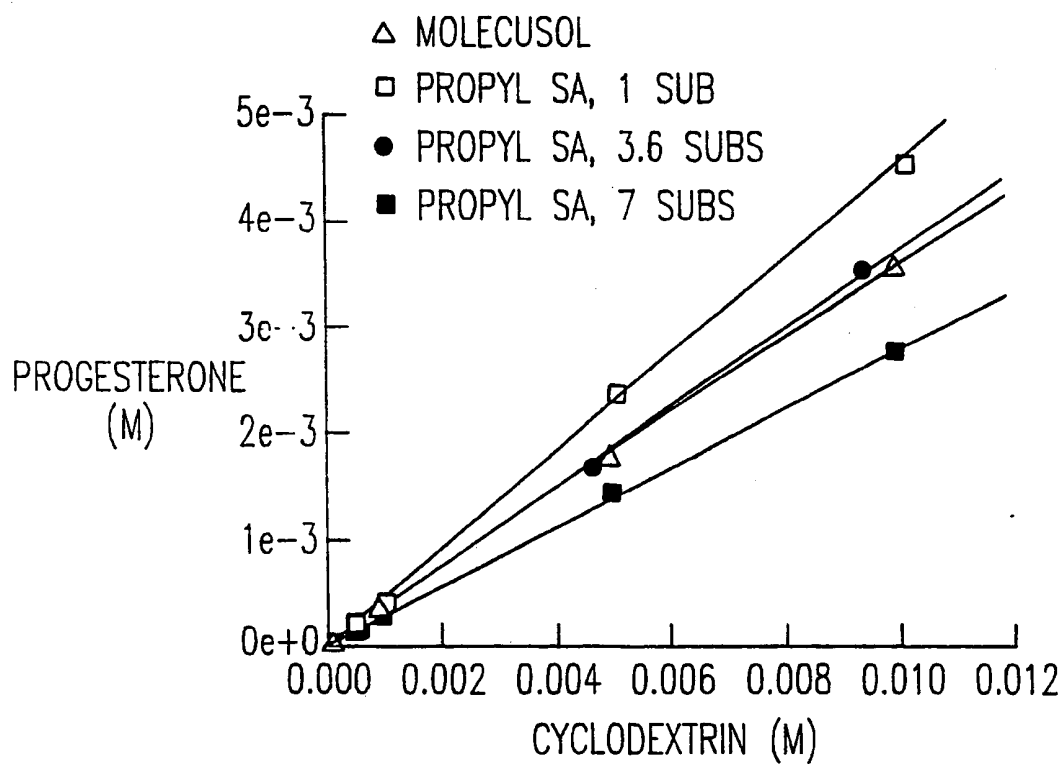


FIG. 2

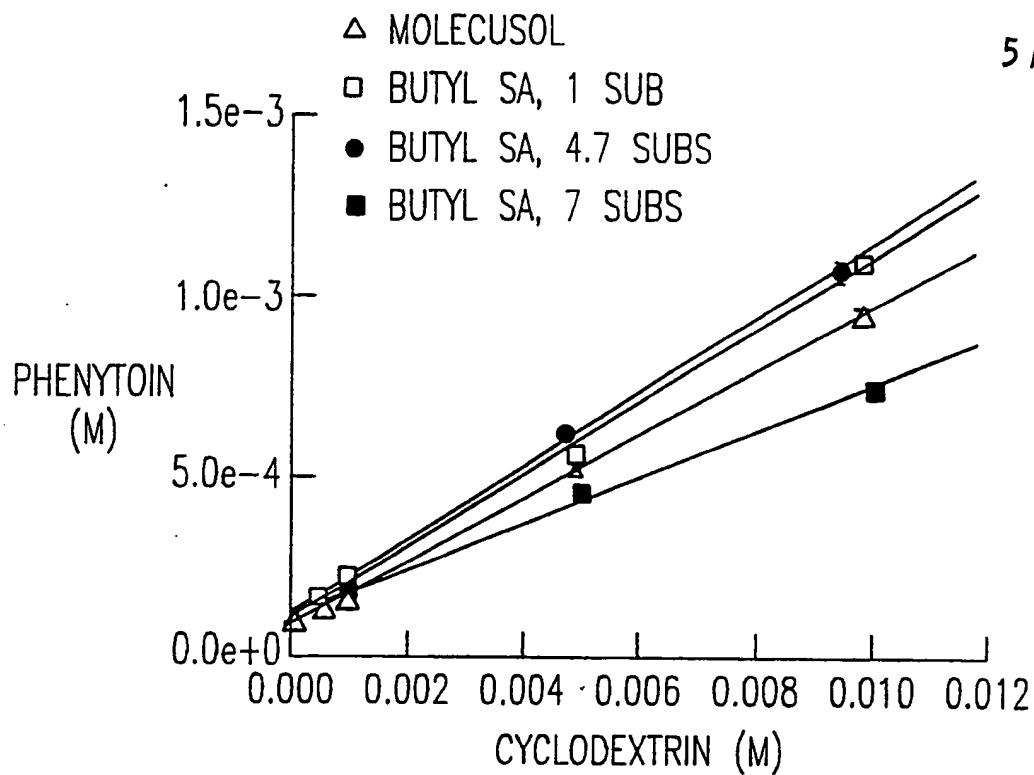
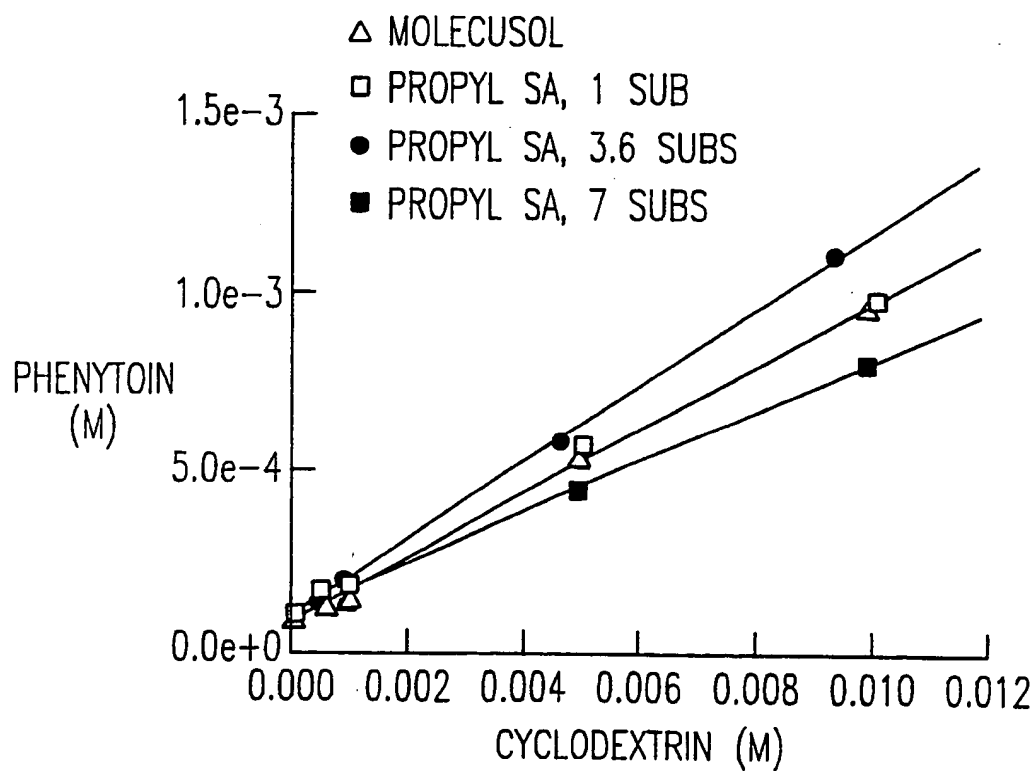
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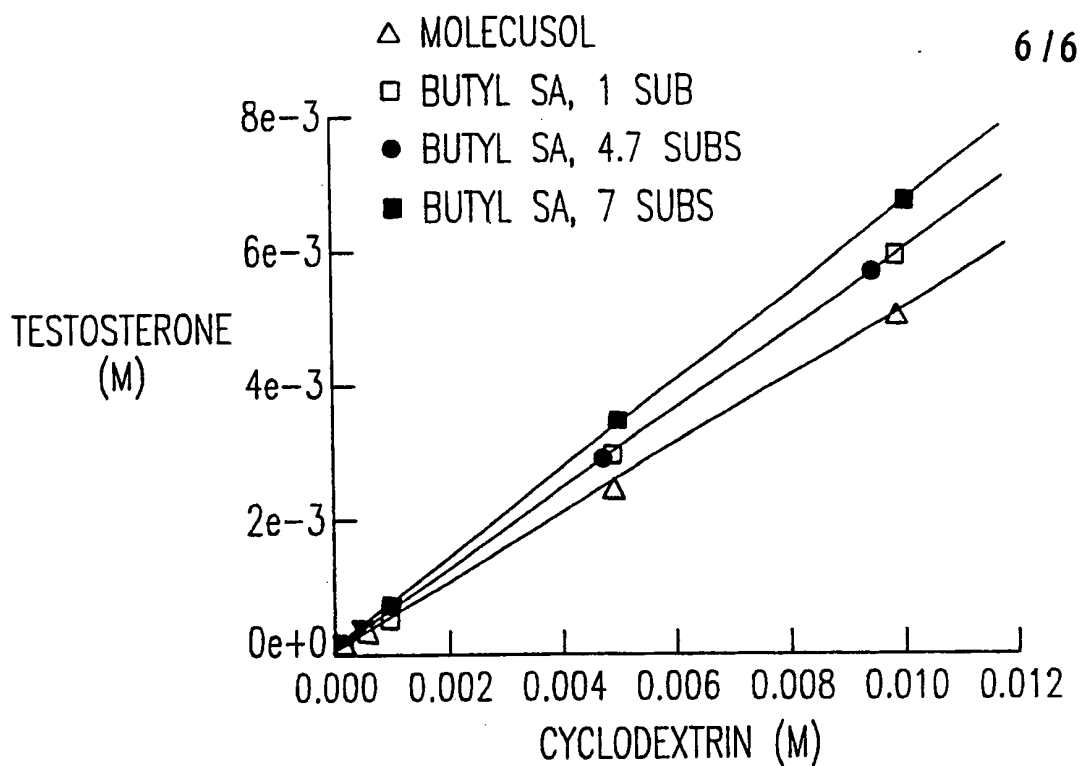
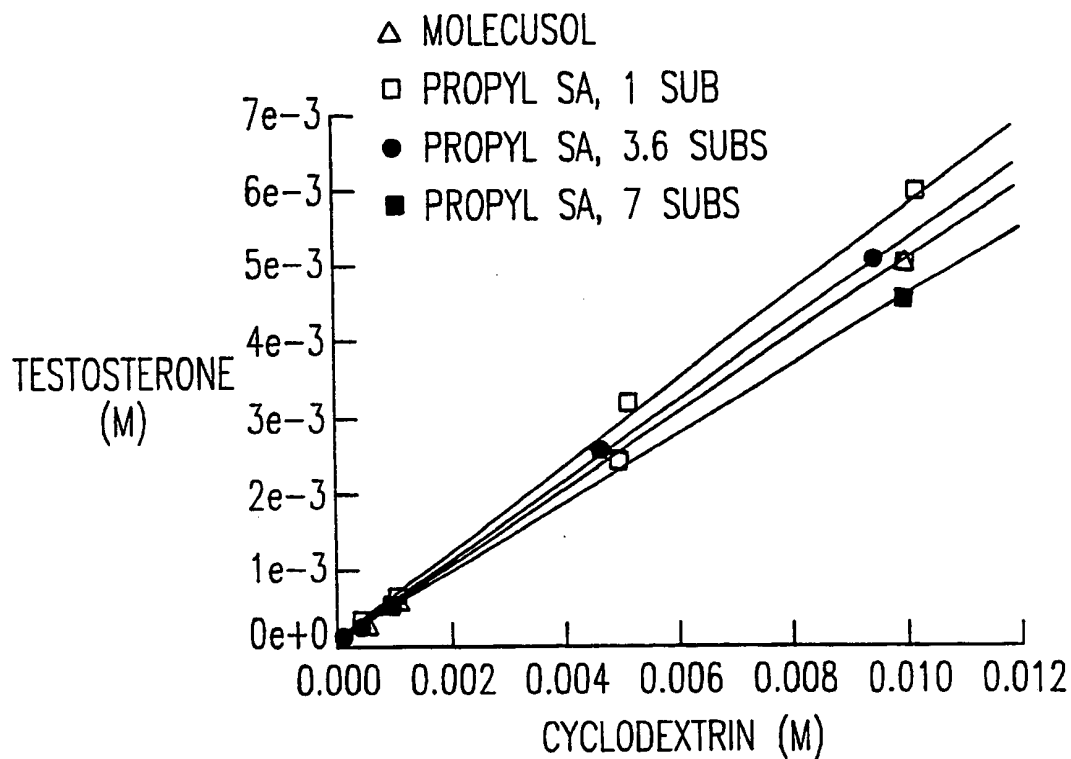
*FIG. 3*

*FIG. 4A**FIG. 4B*

*FIG. 5**FIG. 6*

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*FIG. 7**FIG. 8*

**FIG. 9****FIG. 10**

INTERNATIONAL SEARCH REPORT

International Application No PCT/US91/00326

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 9/00; A61K 47/40; C08B 37/16;

U.S. Cl.: 536/103; 514/58, 778, 964, 965

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System 1

Classification Symbols

U.S. Cl.: 536/103;
514/58, 778, 964, 965

Documentation Searched other than Minimum Documentation
to the extent that such Documents are included in the Fields Searched *

III. DOCUMENTS CONSIDERED TO BE RELEVANT **

Category *	Citation of Document, 14 with indication, where appropriate, of the relevant passages 15	Relevant to Claim No. 16
<u>X</u> Y	US.A 4.535.152 (SZEJTLI ET AL.) 13 August 1985. see entire document.	<u>1-7</u> 8-16
<u>X</u> Y	US.A 4.638.058 (BRANDT ET AL) 20 JANUARY 1987. see entire document.	<u>1-7</u> 8-16
A	US.A 4.869.904 (UEKAMA ET AL.) 26 SEPTEMBER 1989. see entire document.	1-16
<u>X</u> Y	US.A 3.426.011 (PARMER ET AL.) 04 FEBRUARY 1969. see entire document.	<u>1-7</u> 8-16
A	US.A 4.774.329 (FRIEDMAN) 27 SEPTEMBER 1988. see entire document.	1-16
A	US.A. 4.497.803 (HARADA ET AL.) 05 FEBRUARY 1985. entire document.	1-16
A	US.A. 4.808.232 (BEESLEY) 28 FEBRUARY 1989. see entire document.	1-16

* Special categories of cited documents: 13

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search *

Date of Mailing of this International Search Report *

09 APRIL 1991

22 MAY 1991

International Searching Authority *

Signature of Authorized Officer 18

ISA/US

Ronald W. Griffin

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